SHORT COMMUNICATION

Landing Preference of *Aedes albopictus* (Diptera: Culicidae) on Human Skin Among ABO Blood Groups, Secretors or Nonsecretors, and ABH Antigens

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ABSTRACT We demonstrated in this study that blood group O subjects attracted more *Aedes albopictus* than other blood groups (B, AB, and A) but were only significantly more attractive than blood group A subjects in 64 human landing tests. We collected saliva from the subjects and tested it for agglutination inhibition, categorized the subjects into secretors or nonsecretors, and studied mosquitoes' landing preferences for those groups. The mean relative percent landing on blood group O secretors (83.3%) was significantly higher than on group A secretors (46.5%). We also compared the attraction to subjects according to blood groups using forearm skin treated with ABH antigens. Blood group O disaccharide (H antigen) attracted significantly more *Ae. albopictus* than did blood group A trisaccharide (A antigen), and subjects treated with blood group A disaccharide attracted significantly more *Ae. albopictus* than did subjects treated with blood group B trisaccharide (B antigen), but ABH antigens did not, in general, influence the landing preference of mosquitoes among ABO blood groups.

KEY WORDS *Aedes albopictus*, landing preference, ABO blood groups, secretors/nonsecretors, ABH antigens

AN EARLIER STUDY REPORTED that *Anopheles gambiae* (species A) preferred to feed on humans of blood group O (Wood et al. 1972). In contrast, another report compared the blood groups of patients suffering from malaria with those of inhabitants nearby the hospital and found that group A was more frequent in malaria cases than in the controls, while group O was less frequent (Gupta and Rai Chowdhuri 1980). The latter report showed that group A was preferentially bitten by mosquitoes if probability of suffering malaria was not different among blood groups. Some humans secrete substances of blood types on the skin, and nonsecretors are humans who do not. Substances of blood types are oligosaccharides. The secretor (FUT2) blood group locus determines the synthesis of soluble A, B, H, and Lewis b blood group antigens in humans (Kishi et al. 1990). Concerning secretor status, blood group O secretors were reported to be preferred more than O nonsecretors by *Aedes aegypti*, and A nonsecretors were preferred over A secretors (Wood 1976). However, Thornton et al. (1976) challenged those reports and suggested that there was no effect of ABO blood group status on host choice. They suggested that the statistical analyses in the earlier reports were incorrect. In this study, we examined the landing preference among ABO blood groups and among secretors or nonsecretors using proboscis-amputated female *Ae. albopictus*, as previously detailed (Shirai et al. 2000a). We also examined the landing preference for ABH antigens after topical application of oligosaccharides to the forearm skin of subjects.

Materials and Methods

Mosquitoes. A colony of *Ae. albopictus* was maintained in our laboratory at 24 ± 1°C, 60–70% RH, and a 14:10 (L:D)-h photoperiod. The collection site in Japan was Ogaki in the Gifu Prefecture. We used 20- to 30-d-old unfed females after 2–6 generations.

Volunteers and Blood Group Status. We used 64 volunteers (32 males, age, 18–61 yr; 32 females, age, 5–61 yr) as test subjects and established a 30-yr-old male (blood group A, secretor) as a control. Their blood group status was determined initially by a questionnaire.

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Mosquito Landing Tests. We adapted an aquarium (600 by 295 by 360 mm; NS-6M, NISSO, Tokyo, Japan) as a test chamber and introduced 35 mosquitoes into it after amputating their proboscises (Shirai et al. 2000a). The hands and forearms of human subjects were inserted through the sleeve into the test chamber with the backside of the hand up, keeping a small space between the palm and the bottom surface. Mosquitoes landed on the inserted hands and forearms and attempted to bite, but they were unable to feed because their proboscis had been amputated. The number of mosquitoes that alighted on the hands and forearms of subjects was counted every 30 s. Attractiveness was calculated by the total number of landing mosquitoes on an arm, and these 20 counts were added for a 10-min exposure. The hand and forearm of the control host were inserted through the left sleeve of the chamber containing mosquitoes, whereas the hand and forearm of a second subject was inserted through the right sleeve. These were paired comparisons with one test person (who has one arm in the chamber) and the control person (who has one arm in the chamber). An index of attractiveness was derived as percent landings one subject per sum of the subject and the control and repeated three times with naïve mosquitoes. To eliminate the effect of bias toward one side of the bioassay container, the positions of the test and control subjects within the chamber was switched in each succeeding trial. We divided subject's mosquito landings by sum of subjects and controls. Then we multiplied it by 100, and we got the subject's relative percentage of landing. We divided subject's relative percentage by control's, and multiplied it by 50. This value was the subject's index of attractiveness when the control's was 50. For example, when mosquito landings on X subject were 20 and those landing on the control were 30, the relative percentage of landing on X was $20/50 \times 100$ (40.0%) and the relative percentage of landing on the control was $30/50 \times 100$ (60.0%). When mosquito landings on Y subject were 25 and those on the control were 15, the relative percentage of landing on Y was $25/40 \times 100$ (62.5%) and relative percentage of landing on the control was $15/40 \times 100$ (37.5%). The index of attractiveness of X was $40/60 \times 50$ (33.3) and of Y was $62.5/37.5 \times 50$ (83.3). The mean percentage of landings on O, A, B, and AB subjects was analyzed statistically using Fisher's PLSD method by StatView (1998).

Identification of Secretor Status. We collected saliva from 57 (89%) of 64 volunteers and tested the saliva for agglutination inhibition. We preserved saliva at −80°C until it could be tested. After thawing, each saliva specimen was boiled for 30 min and centrifuged at 2,000 rpm for 5 min, and the supernatant saliva was used. We dropped 100 μl PBS (Dulbecco's phosphate-buffered saline; Life Technologies, Grand Island, NY) into each well of one row of a 96-well microtiter test plate. We then dropped 100 μl of one volunteer's saliva into the leftmost well of the top row (row 1), mixed it well, and removed 100 μl of the 200 μl and placed that into the next well over to the right. After repeating this serial dilution 10 times, we produced 10 doses of each saliva sample (diluted every half concentration). Next, we removed three 25-μl aliquots from each of those wells and placed them in the three wells immediately below (rows 2, 3, and 4). We added 25 μl of anti-A serum, anti-B serum (Wako Chemical Co., Osaka, Japan) or anti-H serum (anti-H lecithin from *Ulex europaeus;* Biotest AG, Dreieich, Germany) into each of the 10 wells on rows 2, 3, and 4. We then mixed the plates for 1 h on a vibration table and stored them at 5°C overnight. The next day we removed 25 μl of each diluted saliva sample that had been mixed with one of the three anti-sera and dropped those aliquots into wells of another microtiter plate for agglutination tests. We produced three such plastic microtiter plates per volunteer. Next, we transferred 25 μl of A, B, or O blood cells (reagent red blood cells, 2–4% suspension; Immucor, Norcross, GA) into each well containing saliva/anti-serum, mixed the plates by gentle swirling with our hands, and let them set for 15 min. We judged the agglutination reactions and scored them in four steps: three steps of positive and one step of negative (Kishi et al. 1990). Based on the results of the agglutination test, we judged volunteers whose saliva had a strong agglutination inhibition ability as "secretors" and those that had weak inhibition as "nonsecretors." For secretors, we compared the answers of their ABO blood group in the questionnaire and confirmed each volunteer's ABO blood group.

ABH Antigen Treatment. We washed both forearms and hands of volunteers with flowing water. We treated the washed skin with various concentrations (10^{-6}, 10^{-5}, 0.1, 1, and 10 ppm) of blood group H disaccharide (Fucα1–2Gal), blood group A trisaccharide (GalNAcα1–3[Fucα1–2]Gal), or blood group B trisaccharide (Galα1–3[Fucα1–2]Gal; all antigens were from Dextra Laboratories, Reading, United Kingdom) diluted in 1 ml of distilled water on all parts of one forearm. Blood group H disaccharide is blood group O antigen. One milliliter of distilled water was placed on the other forearm of each subject as a control (C). There were 14 volunteers, which was composed of 2 O secretors, 2 O nonsecretors, 4 A secretors, 3 B secretors, and 3 AB secretors. We compared percent landing of H versus C, A versus C, H versus A, H versus B, and A versus B by paired *t*-test. Each replicate test of 30-s counts for 10 min produced 20 observations.

Results

Landing Preference Among ABO Blood Groups. Among 64 volunteers (type O: $n = 19$, type A: $n = 21$, type B: $n = 17$, and type AB: $n = 7$), the mean relative percentages (±SE) of landings were as follows: O, 78.5 ± 12.4% > B, 56.9 ± 10.0% > AB, 48.0 ± 12.6% > A, 45.3 ± 6.2%. The mean percent landing on blood group O was only significantly higher than that on A ($P = 0.02$, Fisher's PLSD, Fig. 1).

Landing Preference Among Secretors or Nonsecretors. As a result of the agglutination inhibition tests on 57 (89%) of 64 volunteers, the number of secretors or nonsecretors within blood groups was as follows: O

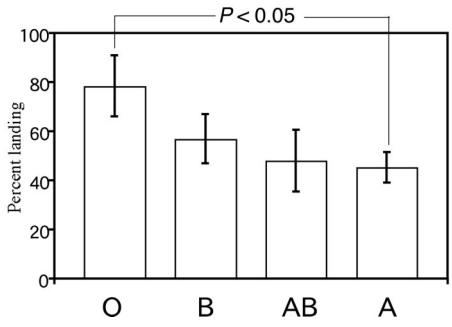

Fig. 1. Landing preference among ABO blood groups by *Ae. albopictus*. Percent landing on O subjects was significantly higher than on A subjects by Fisher's PLSD test ($P < 0.05$). Values represent mean ± SE.

secretors, $n = 13$; O nonsecretors, $n = 4$; A secretors, $n = 17$; A nonsecretors, $n = 1$; B secretors, $n = 14$; B nonsecretors, $n = 2$; AB secretors, $n = 6$; AB nonsecretors, $n = 0$. The mean relative percent landing on blood group O secretors (83.3%) was significantly higher than that on A secretors (46.5%; $P = 0.03$, Fisher's PLSD, Table 1). Although the percent landing on O secretors tended to be higher than on O nonsecretors and that on A nonsecretors tended to be higher than on A secretors, the differences were not statistically significant.

Landing Preference Among ABH Antigens. The H antigen was significantly repellent against the control at 10^{-5} and 1 ppm, but there was no significant difference against the control at 10^{-6} ppm. This result does not show the attractiveness of blood group O at all. The A antigen at 10^{-6} and 1 ppm significantly attracted fewer mosquitoes than the control ($P < 0.0001$ by paired *t*-test). This result is consistent with the lowest preference for blood group A. The H antigen was more attractive than the A antigen, and the A antigen was more attractive than the B antigen. Concerning H versus B, there was no significant difference (Table 2).

Discussion

In our study, we used *Ae. albopictus* as test insects, and we counted the number of landings on the forearm skin of volunteers. Our results differ from a report (Wood et al. 1972) that found a preference for blood group O by checking the blood from mosquitoes (*Anopheles gambiae*) after feeding. However, despite differences in the methods used in these studies, the landing preferences are the same; that is, O > B > AB > A.

Blood group O disaccharide (H antigen) on human skin attracted *Ae. albopictus* more than blood group A trisaccharide in some concentrations, and blood group A trisaccharide repelled *Ae. albopictus* more than the control in some concentrations. However, even the landing tests on ABH antigens do not provide an explanation for the landing preference among ABO blood groups, and there may be other unknown influences underlying the differences of ABO landing preference. In fact, ABH antigens are thought to exist on human skin in low concentrations, and we suppose that mosquitoes cannot perceive them.

In earlier studies, *Ae. aegypti* (Wood 1976) and species A of *An. gambiae* (Wood et al. 1972) exhibited feeding preferences for humans of the O blood group. *Ae. aegypti* is thought to have evolved in Africa (Christophers 1960), and *An. gambiae* is a complex of sibling species that is restricted in geographic distribution to Africa (Coetzee et al. 2000). It is possible that the host preference of these mosquito species for humans of the O blood type evolved because this blood type is highly prevalent in Africa (Mourant and Kopec 1976). In contrast, the origin of *Ae. albopictus* is Asia (Hawley 1988). In Asia, blood type O is not as prevalent as in Africa. For example, in the Miyagi Prefecture of Japan, in a study of 240,204 people, the percentages of ABO blood groups were as follows: O, 32.3%; A, 36.4%; B, 22.8%; AB, 8.5% (Akaishi et al. 1959). Consequently, because of the high prevalence of other blood groups, *Ae. albopictus* did not evolve a preference for humans of type O blood. Certainly, the propensity of *Ae. albopictus* to selectively land on humans based on their blood type is not strongly supported by the results of this study. The effects of blood type on the reproductive capacity of *Ae. albopictus* is presently unknown but warrants future research.

Anopheles gambiae sensu strictu and *Ae. aegypti* are more host-specific for humans than is *Ae. albopictus*. However, the broader host feeding habits of *Ae. al-*

Table 1. Number of landings of *Ae. albopictus on* ABO blood groups and secretors or nonsecretors

Blood group	Subject (S)	Control (C)[b]	Ratio (S/C)	Percent landings	Mean relative percentage of landing[c]	*n*
O secretors	57.75 ± 7.34[a]	41.60 ± 5.31	1.39	58.13	83.32 ± 14.05a	13
O nonsecretors	34.63 ± 7.32	64.88 ± 17.84	0.53	34.80	58.66 ± 40.32ab	4
B secretors	35.31 ± 4.92	32.65 ± 2.88	1.08	51.96	61.67 ± 11.76ab	14
B nonsecretors	40.17 ± 3.50	65.67 ± 15.67	0.61	37.95	31.31ab	2
A secretors	42.15 ± 5.19	53.76 ± 6.37	0.78	43.95	46.49 ± 7.34b	17
A nonsecretors	35.0	25.0	1.40	58.33	62.95ab	1
AB secretors	37.07 ± 7.04	47.74 ± 8.30	0.78	43.71	45.56 ± 14.65ab	6
AB nonsecretors	—	—	—	—	—	0

[a] Values represent mean ± SE.
[b] The control subject was blood type A. secretor.
[c] Different letters on each column show significant difference at $P < 0.05$ by Fisher's PLSD.

Table 2. Number of landings by *Ae. albopictus* on forearms treated with some oligosaccharides or water by *Ae. albopictus* during 10 min

Concentration	Concentration (ppm)	Blood group O (H antigen) disaccharide	Control (water)	df	*t*	*P*	*n*
1 pg/ml	10^{-6}	8.12 ± 0.50[a]	8.23 ± 0.39	99	−0.21	NS	100
10 pg/ml	10^{-5}	3.88 ± 0.25	7.44 ± 0.62	79	−7.294	<0.0001	80
1 μg/ml	1	1.57 ± 0.17	2.52 ± 0.23	119	−4.203	<0.0001	120
Concentration	Concentration (ppm)	Blood group A trisaccharide	Control (water)	df	*t*	*P*	*n*
1 pg/ml	10^{-6}	3.78 ± 0.42	8.25 ± 0.55	79	−2.055	<0.0001	80
10 pg/ml	10^{-5}	4.56 ± 0.35	4.44 ± 0.26	79	0.231	NS	80
1 μg/ml	1	1.08 ± 0.09	1.78 ± 0.15	119	−5.079	<0.0001	120
Concentration	Concentration (ppm)	Blood group O (H antigen) disaccharide	Blood group A trisaccharide	df	*t*	*P*	*n*
0.1 μg/ml	0.1	4.39 ± 0.31	3.58 ± 0.16	239	2.671	<0.01	240
1 μ g/ml	1	4.11 ± 0.23	3.49 ± 0.18	179	2.5	<0.05	180
Concentration	Concentration (ppm)	Blood group O (H antigen) disaccharide	Blood group B trisaccharide	df	*t*	*P*	*n*
0.1 μg/ml	0.1	2.93 ± 0.24	2.97 ± 0.26	119	−0.134	NS	120
Concentration	Concentration (ppm)	Blood group A disaccharide	Blood group B trisaccharide	df	*t*	*P*	*n*
0.1 μg/ml	0.1	3.39 ± 0.22	2.57 ± 0.18	119	3.541	<0.001	120

[a] Values are indicated by means ± SE.
NS, not significant.

bopictus is unlikely to be because of the lack of clear preference among human blood groups exhibited in our study.

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